may play a role in the development of certain genetic diseases. We have selected a guanine-rich portion of the mouse cyclin-dependent kinase 5 regulatory subunit 2 (CDK5R2) mRNA 3' untranslated region for the study of these regulatory G-quadruplexes, as this gene has been implicated in the pathogenesis of a particular inherited disorder, fragile X syndrome (FXS). Circular dichroism spectroscopy, 1H-nuclear magnetic resonance spectroscopy, UV spectroscopy, thermal denaturation experiments, and polyacrylamide gel electrophoresis (PAGE) were utilized to confirm the presence of and characterize G-quadruplex structures in the selected sequence. Electromobility shift assays and fluorescence spectroscopy experiments were performed to demonstrate the binding of CDK5R2 mRNA and the fragile X mental retardation protein (FMRP) arginine-glycine-glycine box domain, which is known to have high binding affinity for G-quadruplex structures.

1193-Pos Board B144

A Conserved Mechanism within the 5'-Leader RNA Genome among HIV-1 and SIVcpz Strains during Viral Genome Recognition

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Previous studies in our lab suggested that HIV-1 5′-Leader (5′-L) RNA genome is regulated by a molecular structural switch. The dimer promoting GC-rich loop of the dimer initiation site (DIS) hairpin is sequestered by base pairing with the unique-5' region (U5) in the monomeric form. The DIS:U5 interaction is then displaced by a stem-loop region containing gag start codon (AUG). The U5:AUG interaction, therefore, occurs and promotes dimerization of the 5′-L. The question raised is whether this structural switch conserved in lentivirus family. Sequence alignment, gel electrophoresis, ITC, and NMR methods are employed in this study to compare RNA constructs from HIV-1, SIVcpz_TAN1, and SIVcpz_US strains. We found both SIVcpz_TAN1 and US strains utilize a similar RNA structural switch to HIV-1 within their 5′-L. This conserved function can be a potential candidate for anti-viral drug development.

1194-Pos Board B145

Site-Directed Spin Labeling Evidence of the Leader-Linker Interaction in the Glycine Riboswitch using Electron Paramagnetic Resonance Spectroscopy

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Riboswitches are mRNA transcripts that function to modulate genetic expression through selective recognition and binding of cognate ligands which, subsequently, induces RNA conformational changes. Continuous wave electron paramagnetic resonance (CW EPR) spectroscopy, when employed with site-directed spin labeling (SDSL), is a useful technique for investigating changes in site-specific dynamics within biological systems. In this work, SDSL CW EPR was used to study the leader-linker interaction in the Vibrio cholerae glycine riboswitch. The glycine riboswitch binds two molecules of glycine to regulate the expression of genes associated with glycine metabolism. The recently described leader-linker interaction in the glycine riboswitch has been investigated using biochemical methods and was shown to play a functional role in the ligand binding process. To probe local RNA backbone dynamics of select sites within the leader-linker interaction SDSL, using the R5 spin label, was employed. Incorporation of spin labels was achieved through the use of optimized ligation methodologies that allow small, synthetically modified RNA to be joined to the larger riboswitch RNA sequence. Empirical analysis of X-band EPR line shapes was used to characterize dynamics of the interaction at varying temperatures for differing folded states of the riboswitch in the absence or presence of salts and glycine ligand. Spectral variation at the labeled sites is in agreement with postulated secondary structural elements and has provided spectroscopic evidence in support of the leader-linker interaction.

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Structure of Adenovirus Virus Associated RNA-I

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Protein kinase R (PKR) is activated by dsRNA produced during virus replication and plays a major role in the innate immunity response to virus infection. In response, viruses have evolved multiple strategies to evade PKR. Adenovirus virus-associated RNA I (VAI) is a short, non-coding transcript that function as an RNA decoy to sequester PKR in an inactive state. VAI consist of an apical stem-loop, a highly structured central domain, and a terminal stem. Chemical probing and mutagenesis experiments were used to produce a refined secondary structure of VAI and to probe tertiary structure within the central domain. Nucleotides within two loops are protected from SHAPE modification, indicating that they participate in tertiary interactions. Mutations within either loop designed to disrupt a putative pseudoknot result in enhanced modification at both sites. Introduction of compensatory basepairs induces protection, indicating that these loops interact to form a pseudoknot. The residue-level constraints from SHAPE and low-resolution structural information from SAXS measurements were used to develop an atomic model of VAI. The ab initio model of VAI derived from SAXS measurements contains a central bulged region flanked by a short arm and a longer, kinked arm. A putative atomic model of VAI was predicted based on the experimental constraints from SHAPE analysis using a fragment assembly approach. The atomic structure agrees well with the SAXS envelope. This model rationalizes the roles of the three domains of VAI in mediating high affinity PKR binding.

1196-Pos Board B147

Small Fdu Strand Exhibits Salt-Dependent Stability

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As shown in our previous work, F10 (a 10mer of Fdu - 5-fluoro-2′-deoxyuridine-5′-O-monophosphate) has a 40% likelihood of folding into a stable hairpin structure when Magnesium ions are used to neutralize the simulated system. Investigations of other counter ions revealed that F10 folds less than 10% of the time when neutralized with ions with a +1 charge and 40% of the time when neutralized with ions with a +2 charge. When simulated with biologically relevant combinations of K^{1+} , Na^{1+} and Mg^{2+} ions, F10 folds into this hairpin structure approximately 10% of the time; however, once the strand of Fdu enters this folded state, it is 96% likely to stay in this kinetic trap. Here we present the data from microsecond timescale simulations and hypotheses on F10's behavior in the presence of various counter ions.

1197-Pos Board B148

Molecular Simulations Studies of RNA Tetraloop Hyperstability: The Effect of Stem Length on Folding Dynamics

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RNA hairpins, formed by looping oligonucleotides, are among the most common RNA secondary structures and involve conserved interactions that drive hierarchical folding and stabilize folded states. Recent modifications to all-atom molecular dynamics force fields have provided the means to quantitatively assess RNA hairpin folding and stability for comparison with experiment. This study focuses on using these techniques to analyze two hyperstable RNA hairpins, each containing a hyperstable tetraloop sequence (rGCAA) and a G-C stem of two or four basepairs. The systems were simulated for a total of 300 microseconds using replica exchange molecular dynamics, and the resulting structure ensembles were analyzed for common folded states, intra-loop hydrogen bonds, and stem base-pairing retention. We find that the longer stem produces kinetic traps with non-native loop conformations, while offering credence to supposed alternative tetraloop folds from experiment.
Shorter stems produce more variable folding behaviors and demonstrate reversible folding/unfolding actions. These results offer an unbiased, thermodynamic characterization of RNA tetraloops, highlight the presence of kinetic traps, and provide critical information for kinetic folding studies of tetraloops as well as additional folding studies of larger RNA molecules.

1198-Pos Board B149

Sequence Specificity in RNA-Mediated Transcriptional Attenuation Examined by Coarse-Grained MD Simulations

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In RNA-mediated transcriptional attenuation, the elongation of a nascent mRNA is halted prematurely through the binding of a non-coding, antisense RNA. This process is initiated by a kissing loop complex between the antisense and target hairpins. Complementarity then favors extended inter-strand base pairing; however, transcriptional attenuation requires rapid kinetics, which depend highly on sequence. Computational prediction of sequences that rapidly form extended complexes would aid in the rational design of mutually orthogonal regulators, allowing their simultaneous application to synthetic systems without generating crosstalk. Additionally, probing the energetics of these systems provides insight into the fundamental relationship between RNA sequence, structure and function.
Here, we employ coarse-grained molecular dynamics (MD) simulations to investigate the potential of synthetic RNA hairpins, derived from prokaryotic attenuation systems such as pT181, to form extended complexes with their antisense partners. The resulting trajectories of an experimentally known RNA-attenuator